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THE PENICILLIUM LUTEUM-PURPURO- GENUM GROUP¹

CHARLES THOM

Wehmer² in a recent paper pointed out what he believes to be natural cleavage lines among the species commonly lumped together under the generic name of *Penicillium*. In that paper he reasserts the validity of his separation of *Citromyces* as a separate genus, transfers the coremiform species *P. claviforme* and *P. silvaticum* to the genus *Coremium* Link, incorrectly attributed to Corda, and revived for the purpose, and indicates the desirability of separating *P. brevicaulis* and its allies without recognizing that this was done by Bainier³ in 1907 under the name of *Scopulariopsis*.

The validity of the latter group has been amply confirmed by the work of Miss Dale,⁴ whose cultures have been seen by the writer of this paper.

The application of the name *Coremium* to *P. claviforme* Bainier and *C. silvaticum* Wehmer is appropriate. Whether the botanical rules of nomenclature will permit such use is, however, very doubtful from the history of the name *Coremium* as given elsewhere.⁵

If the *Coremium* group were limited to the two species named, separation would be easy. However, *P. duclauxi* Delacroix

¹ Published by permission of the Secretary of Agriculture. This paper is a revision and extension of a paper read before the Botanical Society of America in Philadelphia, in December, 1914, under the title "The *Penicillium* Group Verticillatae of Wehmer."

² Wehmer, C. Ber. deut. Botan. Ges. 32 (1914), Heft. 5, pp. 373-384. *Coremium silvaticum* n. sp. nebst. Bemerkungen zur Systematik der Gattung *Penicillium*.

³ Brainier, G. Bul. Soc. Myc. France, 23 (1907), p. 98.

⁴ Dale, E. On the fungi of the soil. Ann. Mycol. 12 (1914), No. 1, pp. 33-62.

⁵ Thom, C. Cultural Studies of Species of *Penicillium*, U. S. Dept. Agr. Bur. Anim. Ind. Bul. 118, Washington, 1910.

under some conditions of culture falls definitely within the *Coremium* group as used by Wehmer. Under other conditions it becomes a simple *Penicillium*.⁶ *P. expansum* Link, observed upon a rotten apple, is usually typical for the genus *Coremium*, but when transferred to culture media becomes a *Penicillium* again, which under many cultural conditions shows no sign of coremia.

Sopp⁷ has also definitely broken the penicillately fruiting organisms into several genera upon morphological lines.

The difficulties encountered in the use of either plan suggest the desirability of continuing the general use of the name *Penicillium* for the entire group until definite natural lines of cleavage can be established.

Penicillium, in the narrowest sense, as discussed by Wehmer, includes those forms which produce green mold surfaces consisting of a large number of separate conidiophores. Within this narrowest group he finds also natural cleavage lines which perhaps represent real relationship. He has pointed out one such natural group and suggested that it be called the *Verticillatae*. This section and a single series within this group form the subject of this paper. In the past ten years about fifteen members of this series have been collected from widely separated regions. Some of them, *P. luteum*, *P. africanum*, *P. pinophilum*, and *P. purpureogenum* were already named and described without indication of relationship. Several, for example, *P. africanum* and *P. purpureogenum*, among named forms, are closely enough related to make separation troublesome even to the describer.⁸ When several other members of the series are added, the description of each in terms which will insure identification by the next worker becomes more difficult, perhaps impossible. Descriptions and figures for a series of these forms were prepared, laid away, then reread, and compared along with fresh cultures and accumulated cultural data. Some of these forms have been kept in continuous culture for about ten years. They maintain the individuality of old friends. Such strains are well established biological entities but to draw a tech-

⁶ Thom, C. l. c.

⁷ Sopp, O. Johan-Olsen. Monographie der Pilzgruppe, *Penicillium*, etc. Videnskapsselskapets Skrifter I. Mat.-Naturv Klasse 1912, No. 11, Christiania, 1912.

⁸ Doebebt, H. Ann. Mycol. 7 (1909), No. 4, pp. 315-338.

nical characterization which will surely separate them is exceedingly difficult. The common morphological characters have, therefore, been brought together to define the section *Verticillatae* of the whole group of penicillate organisms in the sense of Wehmer. These characters follow:

MORPHOLOGICAL CHARACTERS OF SECTION VERTICILLATAE

Conidiophores vary greatly in length. Each conidiophore typically produces a crown or verticel of metulae,⁹ or fertile branches. From this arrangement Wehmer derives the name *Verticillatae*. The metulae are few to many in the verticel; the number differs in the different members of the series. They are usually closely clustered. Careful search in nearly every strain shows an occasional conidial fructification in which a secondary verticel is borne either upon the main conidiophore prolonged through the center of the primary verticel or upon some one of the metulae of the primary series. Both conditions are found, but only in small numbers under the usual conditions of culture.

This section includes many species, some of which form series in which further relationship is suggested. Others diverge widely. One such series of forms has been collected and may be designated the luteum-purpurogenum series from the well known forms which stand at the extremes.

THE LUTEUM-PURPUROGENUM SERIES

At one end of this series stands a strain of *P. luteum*¹⁰ Zukal which produces ascospores freely in all the media used and conidia very sparingly. In the actively growing culture the dominant shades of color are yellow with tardy appearance of red. At the other end stands *P. purpurogenum* Stoll,¹¹ which produces only conidia, in which yellow shows transiently, while red colors in mycelium and substratum are abundant.

⁹ Westling, R. Arkiv. för Botanik. Bd. 11, No. 1, p. 44, in Ueber die grünen spezies der Gattung *Penicillium*.

¹⁰ Described as *P. luteum* in Bul. 118, B. A. I. U. S. Dept. Agr. The culture was cultivated by Prof. R. Thaxter in 1905.

¹¹ Stoll, O. Beiträge zur Morphologischen und biologischen Charakteristik von *Penicillium*, Inang. Dissert. Stuttgart 1903-4.

Among these forms is one well-marked organism (No. 2670) *P. purpurogenum* var. *rubri-sclerotium*, with all the common characters of the series but producing abundant sclerotia, dark-red to black in color, upon the surface of the substrata. If these sclerotia should be found to be undeveloped perithecia, the form would be clearly eliminated from genetic relationship to the forms at the luteum end of the series, but would probably take with it the other forms at the purpurogenum end of the series.

COLONY CHARACTERS

The production of yellow in the surface growth at some period of colony development or under some cultural conditions is typical for the group. This may be dominant, transient, or almost lacking yet it is not difficult to demonstrate in the organisms studied. Microscopic examination shows this color to be due to the encrustment of the aerial hyphae, or part of them, with yellow granules. Definite quantitative differences in this color are shown by successive cultures of the same strain in different media, especially in media with differing reaction. The different numbers of the series show fairly constant differences in the amount of yellow color produced. This quantitative difference is partly at least due to the characteristic differences in the amounts of surface mycelium produced by the different races. Color increases with floccosity. In *P. pinophilum*, Hedgecock¹² found that the color of the granule was yellow when acid, and a reddish shade when alkaline. In the strain of *P. luteum*, previously described (Bul. 118, Bur. Anim. Ind. U. S. Dept. Agr., No. 11), the yellow is a dominant factor during the early growth of the organism, giving place but partially to reddish hyphae in age. The whole culture gets its color from the yellow granules.

The descriptions of Wehmer¹³ indicate the production of conidia much more abundantly by his strain of this species. The subsequent discovery of ascospores conforming to the descriptions of *P. luteum* in other American strains suggested the relationship of this whole series. The ascus-producing forms when brought together show a progressive loss in ascus producing

¹² Hedgecock, G. G. Missouri Bot. Garden, Rept. 17, pp. 105-107.

¹³ Wehmer, C. Ber. deut. Botan. Gesellsc. 1893, p. 499.

power with progressive development of large conidial areas until we reach forms with few ascigerous masses. From these to the forms with no asci is the next natural step. This transition is suggested by the general morphology of certain forms on which asci have been produced in culture. In every member of the series careful observations show at least a narrow fringe of hyphae studded with yellow granules, about the margin of the developing colony. The yellow is quickly covered by the mass of green spores but usually may still be seen with the microscope.

COLOR IN THE SUBSTRATUM

Coincident with the change of color in the surface or aerial growth we find at the luteum end of the series that yellow to orange shades predominate in the substratum. These slowly or but partially change to red as the colonies become old. In the forms producing conidia only, yellow or orange tones still appear in the young colony. The change to red is slow and only partial in some forms but towards the purpurogenum end of the series the yellow colors are reduced to but transient appearances, replaced quickly and almost completely by red. Observations upon these changes must be repeated at intervals during a period of two to three weeks.

MORPHOLOGY

The members of this series show the conidiophore character of the whole section. Together with color production, however, they display an essential uniformity in sterigmatal and conidial characters, shared with some but not all the forms showing the conidiophore and branching described by Wehmer for the *Verticillatae*.

STERIGMATA

Each branch or metula bears at its apex a closely packed cluster of sterigmata¹⁴ (syn. basidia, conidiiferous cells) or conidium-bearing cells. These are closely packed and continue as nearly parallel as mechanical conditions permit. They widen gradually

¹⁴ The use of the term sterigmata here follows Westling who has discussed this series of terms fully, l. c., p. 47.

from the base upward for 5 to 8 μ , then taper slowly to the diameter of the conidium producing tube,¹⁵ with a total length of from 10 to 15 μ . The length of these cells, their lanceolate or lance-acuminate form and closely parallel arrangement is characteristic for the whole group.

CONIDIA

The conidia arise as cylindrical cells which change usually through fusiform to elliptical or in some cases almost globose, with walls, when fully ripe, either smooth or delicately roughened, or sometimes both in the same culture. The fusiform to elliptical shapes are most common. Variation in size in the same strain and usually in the same culture is very marked. Even strains with conidia averaging small have some which reach the sizes shown by the larger spored forms. Very large single spores and chains of spores are abundant in some strains while rare, or perhaps absent in others. In forms where the conidia have been germinated under observation the conidia swell to these large sizes while germinating. Masses of conidia where present give some shade of green to that part of the colony.

It is, therefore, possible to bring this group together in such a way as to aid the worker in locating the strains found. Whether any one can so define the appearances of each of the separate strains as to ensure identification by others is doubtful. Perhaps for most purposes it is immaterial.

A synoptical arrangement of the strains considered in this paper has been prepared from cultures grown in Czapek's solution agar.¹⁶ When incubated at 37° C. five strains failed to grow, namely, Nos. 2647, 3525.15, 3523.4y, 2751, and 4010.9. The other strains grew well at blood heat. The cultures which failed

¹⁵ Thom, C. Conidium production in *Penicillium*. *Mycologia* 6 (1914) No. 4, pp. 211-215.

¹⁶ Distilled water 1,000 c.c.
 Magnesium sulphate 0.5 gram
 Dipotassium phosphate (K_2HPO_4) 1.0 "
 Potassium chloride 0.5 "
 Ferrous sulphate 0.01 "
 Cane sugar 30.00 grams
 Agar 15.00 "

to develop at 37° C. grew freely as soon as the temperature was lowered. These five strains were distributed through the luteum section of the group; all of the purpurogenum section grew well at blood heat. The arrangement of the forms in the series presents their natural relationship as far as such relationship is determinable. The inclusion and placing of *P. duclauxi* is more or less arbitrary and perhaps due only to superficial evidence. The correlation of colors, color changes, and morphology suggest that the other strains may be safely grouped about *P. luteum*, *P. rugulosum*, *P. pinophilum*, and *P. purpurogenum* without offering specific names at present for new species.

SYNOPSIS OF THE SERIES

*A.*¹⁷ Growing colonies with prominently yellow areas and reverse yellow to orange slowly replaced by reds—Luteum Section.

B. Ascigerous masses found.

a. Ascigerous masses abundant, conidial fruits few, scattered; colonies when young predominantly citron-yellow to strontium-yellow (Ridgway XVI), becoming partially and tardily pale-flesh-color or flesh-color (Ridgway XIV); submerged mycelium and agar (reverse of colony), shading from yellow toward orange-red or red. *P. luteum* Zukal.¹⁸ Cultures No. 11 from¹⁸ Prof. Thaxter, Cambridge, Massachusetts, No. 3522.3 by Dr. J. R. Johnston from Puerto Rico soil.

aa. Ascigerous masses few, green conidial areas well developed with conidiophores more or less in tufts. Yellow color predominates at edge and about ascigerous masses. Reverse colors from yellow toward orange and red. Culture, Thom, No. 2751, sent by Prof. M. T. Cook, from New Jersey.

BB. Ascigerous masses not found.

b. Colonies with sterile areas citron or strontium-yellow and conidial areas changing to flesh-color in age, shading with the amount of green conidia from sea-foam or chartreuse-yellow through citron, lime or chrysolite-green to densely conidial areas near ivy-green (Ridgway XXXI).

c. With conidial areas in scattered tufts; culture from rotting apple.¹⁹ Thom, No. 3525.95, Washington, D. C.

cc. With large more or less irregular green areas. Cultures 3525.15 from rotting apples, Washington, D. C.; Thom No. 4010.9 from Seattle, Washington.

¹⁷ Correlative divisions are indicated by duplication of index letters *A*, *AA*; *c*, *cc*, etc.

¹⁸ *P. luteum* Zukal, Sitzber. K. Akad. Wiss. (Vienna) Math. Naturw. Kl. XCVIII, p. 521, 1889. Cultural description, Thom, C., U. S. Dept. Agr., Bur. Anim. Ind. Bul. 118, p. 39.

¹⁹ Organisms from rotting apples were contributed by Mr. Brooks of the Bureau of Plant Industry.

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ccc. With yellow area restricted to a narrow zone shading quickly to ivy-green.

Culture Thom No. 3523.4y from Virginia soil.

bb. Colonies showing yellows above only in age, in reverse slowly but deeply orange to red. Conidia rough.

d. Without coremia—*P. rugulosum*.²⁰

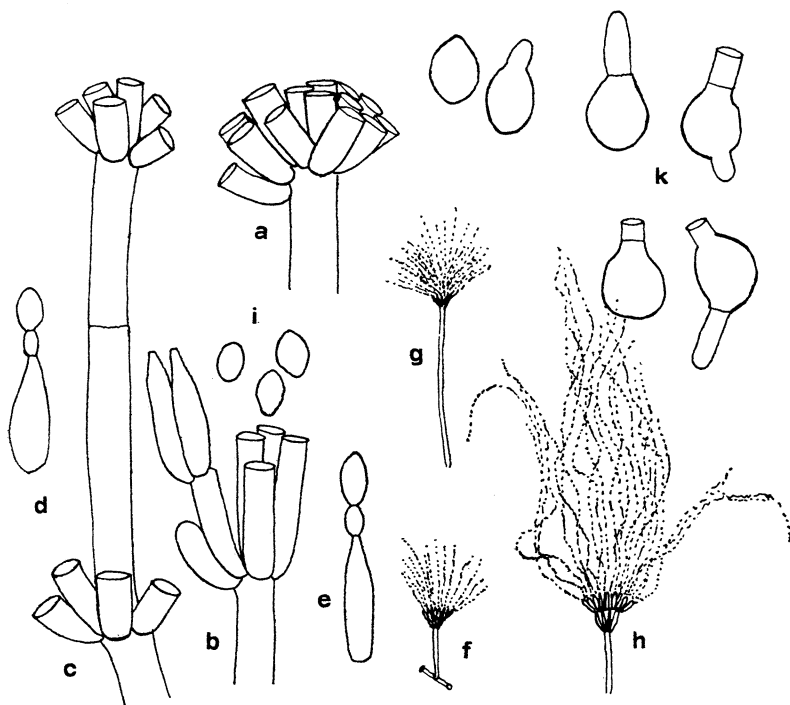


FIG. 1. *Penicillium purpurogenum* var. *rubri-sclerotinum*. a, b, typically verticillate branching at the apex of the conidiophore; c, in this conidial fructification the main axis is prolonged to bear a secondary verticillate above the first; e, d, conidia bearing cells or sterigmata; i, conidia; f, g, h, diagrammatic representation of the entire conidial apparatus; k, germination of conidia. The morphology represented here shows the essential features of the entire group. In some the conidial chains are massed into solid columns, in others as in this divergent.

dd. With numerous long coremia—*P. duclauxi*.²⁰

AA. Conidial areas prominent from the first. Reverse colors predominantly red. *Pinophilum-purpurogenum* section.

C. Colonies develop yellow and green areas together, which remain more or less persistently mixed.

²⁰ Thom, C., l. c. p. 60 and p. 42. The relationship of these organisms to each other and to the other members of the group may only be superficial but this necessitates their citation here.

- e.* Conidia rough; cultures 2647 and 2733 from English soil²¹ and 2500 from New York soil.
- ee.* Conidia smooth, yellow hyphae becoming reddish in age, reverse and agar from salmon-orange to mahogany-red.
- f.* Floccose or tufted. *P. pinophilum* Hedgcock.²² Numerous cultures obtained from soil and from food products can not be separated from this species.
- ff.* Velvety, broadly speaking. Thom No. 43.
- CC.* Growing colonies with margin variously changing through sea-foam green, chartreuse-yellow, or chrysolite-green (Ridgway XXXI), replaced by conidial areas toward celandine-green or andover-green (Ridgway XLVII). Reverse in reds toward oxblood-red (Ridgway I). Deep-red sclerotia developed in age. *P. purpureogenum*, var. *rubri-sclerotium*, var. nov.²³ This organism has been obtained from many sources and widely separated sections of the United States.
- CCC.* Colonies showing little or no yellow at first, yellow hyphae appear frequently in secondary growths in the centers or margins of older colonies. Reverse colors intensely red.
- g.* Conidiophores mostly borne as branches from interlacing aerial hyphae and ropes of hyphae.
- h.* Conidial fructification typically verticillate. *P. africanum* Doebelt, Ann. Mycol. 7 (1909) No. 4, pp. 315-338.
- hh.* Conidial fructifications branched several times. *P. funiculosum*, Thom, l. c., p. 70.
- gg.* Conidiophores arising as branches from separate hyphae. Complex ropes not found.

P. purpureogenum O. Stoll.²⁴

Cultures have been found from American sources, especially several from corn (*Zea Mays*) which cannot be separated by satisfactory marks from the original strain distributed by Kral. The species is probably cosmopolitan.

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²¹ Contributed by Miss E. Dale, Cambridge, England.

²² Thom, C., l. c. p. 31.

²³ *P. purpureogenum* var. *rubri-sclerotium*, var. nov. Differs from typical description of the species in the production of dark-red sclerotia on the surface of the substratum and in the well marked zone of yellow at the margin of the growing colony. It appears to be a soil fungus with well marked characters.

²⁴ The nomenclature of this form is discussed by Thom, l. c. p. 36.